Volume 15 Number 6 1987 Nucleic Acids Research

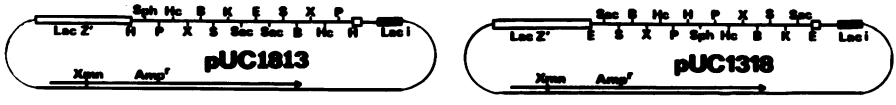
Hybrid pUC vectors for addition of new restriction enzyme sites to the ends of DNA fragments

Robert Kay[1,*] and Joan McPherson[2]

[1]Department of Chemistry, Washington State University, Pullman, WA 99164-4660, USA and
[2]Department of Botany, University of British Columbia, Vancouver, BC, Canada

Submitted January 19, 1987

The most commonly used method for adding new restriction enzyme sites to the ends of a DNA fragment involves attachment of synthetic linker or adapter oligonucleotides by ligation, exposure of the new sites by restriction enzyme digestion, and insertion of the modified fragment into a compatible site in a cloning vector. New flanking sites can also be obtained by directly inserting a DNA fragment into one of the multiple sites in the polylinker region of a vector such as pUC. Insertion into an asymmetric polylinker results in an asymmetric distribution of flanking sites; e.g. insertion into the HincII site of pUC13 places HindIII and PstI sites on one end of the insert and XbaI BamHI, SmaI, SacI and EcoRI sites at the other end. While such an arrangement may be required in some cases, it is more often necessary to add identical sites to both ends of a DNA fragment. Vectors bearing symmetric polylinkers can be used for this purpose. We have constructed a pair of hybrid pUC vectors with symmetric polylinkers centred on unique EcoRI or HindIII sites. They were made by ligating together the 837 bp XmnI-EcoRI fragment of pUC18 and the 1890 bp EcoRI-XmnI fragment of pUC13 (pUC1813), or the 825 bp XmnI-HindIII fragment of pUC13 and the 1902 bp HindIII-XmnI fragment of pUC18 (pUC1318). A DNA fragment inserted into these vectors between one pair of sites becomes flanked by pairs of the remaining sites in the polylinker; e.g. insertion between the HincII sites of pUC1318 results in flanking pairs of XbaI, BamHI, SmaI, SacI and EcoRI sites. Both pUC1813 and pUC1318 retain the colour selection for insert cloning of the parent pUC vectors because, with the single exception of SmaI, removal of the polylinker DNA between paired sites without insertion of extraneous DNA does not shift the reading frame of the Lac Z'-encoding transcript. Symmetric polylinker vectors with different repertoires of sites can be assembled from other combinations of pUC vectors or related vectors containing more complex polylinkers. A few of these vectors can substitute for large and expensive collections of oligonucleotide linkers and adapters.



Vector Structure. The polylinker is expanded for clarity. B, BamHI; E, EcoRI; H, HindIII; Hc, HincII; K, KpnI; P, PstI; S, SmaI; X, XbaI.

*To whom correspondence should be addressed